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Screening Sartopure® PP3 Filter Combinations for Efficient Lentiviral Vector Clarification

Suneetha Nunna, Marcel Fueger, Lucas Herbst, Axel Thiefes, Alexander Tappe
Sartorius Stedim Biotech, Otto-Brenner-Str. 20, 37079 Göttingen, Germany

Contact: Suneetha.nunna@sartorius.com, marcel.fueger@sartorius.com

Abstract

Given the clinical success of lentivirus-based cell and gene therapies, optimizing downstream processing of lentiviral vectors (LVs) is essential to improve manufacturing efficiency. A critical step in this process is the clarification of crude LV harvests, which aims to remove cells and cellular debris while preserving viral infectivity.

This study evaluated the performance of a two-stage depth filtration train for LV clarification, consisting of two Sartopure® PP3 filters with varying retention ratings followed by a Sartopore® 2 filter. Configurations were compared with respect to filter load capacity, turbidity reduction, and preservation of LV infectivity.

All tested filter combinations maintained comparable LV infectivity, with only minor differences in turbidity reduction. However, filter capacity increased with decreasing retention rating of the primary filter. Consequently, primary clarification with an 8 µm Sartopure® PP3 filter, followed by secondary filtration with a 1.2 µm or 0.65 µm filter, outperformed configurations using 20 µm and 50 µm primary clarification filters paired with comparable secondary clarification filters.

Introduction

Lentivirus-based cell and gene therapies are increasingly established in clinical development and therapeutic applications, driving the need for fast, reliable, and efficient manufacturing processes. A typical lentiviral vector (LV) production workflow, as illustrated in Figure 1, begins with HEK293 cell expansion followed by plasmid transfection to initiate LV production. Once sufficient titers are achieved, viral particles are harvested and subjected to downstream processing, typically comprising clarification, purification, buffer exchange, sterile filtration, and final formulation.[1,2]

Following clarification, chromatography provides the main purification step for the removal of process- and product-related impurities, such as host cell proteins and host cell DNA. Depending on the process design, a tangential flow filtration (TFF) step may then be employed to concentrate the product further and exchange it into the final formulation buffer prior to sterile filtration and fill–finish. Each process step is monitored using appropriate analytical methods to ensure effective performance and consistent product quality.[3-5]

As the initial downstream unit operation, clarification plays an essential role in preparing the harvested material for later purification steps. Clarification consists of primary and secondary steps employing filters with decreasing retention ratings.[6] While primary clarification removes cells and coarse cell debris, secondary clarification eliminates finer particulate matter and aggregates. To prevent clogging and ensure efficient performance of downstream purification operations, including chromatographic separation, a microfiltration step is routinely implemented. This is generally accomplished with membrane-based microfiltration devices rated at ≥ 0.45 µm, such as those from the bioburden reduction filter series.[7] Together, these early downstream steps establish the basis for efficient downstream purification, high product recovery, and robust process performance.

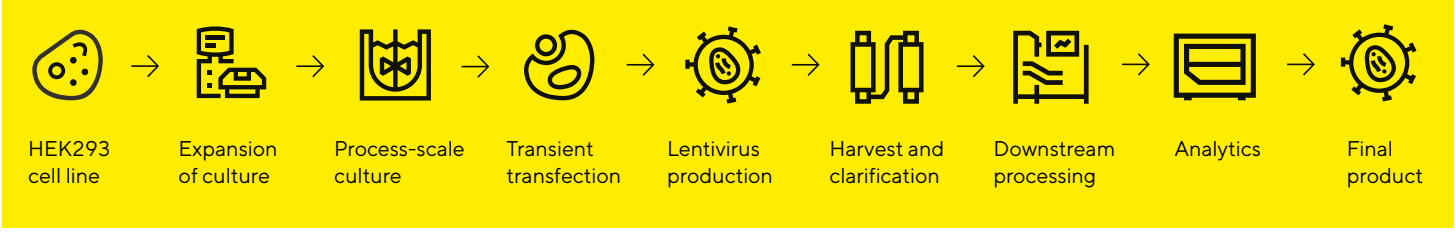
Clarification of complex biological fluids commonly relies on sequential filtration concepts, combining depth-based pre-filtration with a final membrane filtration step. Sartopure® PP3 filters are designed for depth-based prefiltration, enabling efficient removal of cell-derived debris and coarse particulates during clarification of complex biological fluids. Their fleece depth structure supports high retention of solids and stable filtration performance across a wide range of process conditions.

Sartopure® PP3 filters are composed entirely of polypropylene, providing broad chemical compatibility with solvents, acids, and bases, as well as a low extractables profile. The all-polypropylene construction of the Sartopure® PP3 filter enables sterile, fully closed processing, ensuring both process integrity and operator safety. Sartopure® PP3 filters are available in nine different sizes, ranging from 0.012 m^2 capsules to 1.95 m^2 cartridges, as well as the Maxicaps® MR system, which combines nine capsules, enabling straightforward scaling of filtration surface area from small-scale development to large-scale applications.[6]

This application note presents an evaluation of Sartopure® PP3 filter train combinations for primary and secondary clarification of LVs. Filter trains with different retention ratings were compared based on overall filter capacity, turbidity reduction, and infectious titer recovery. Subsequent studies will address filter conditioning using different buffer solutions, bioburden reduction filter screening, and integration of the results into a filter benchmarking study.

Different combinations of two Sartopure® PP3 prefiltration units connected in series were evaluated, with 50, 20, or 8 µm filters used for primary clarification combined with 1.2 or 0.65 µm filters for secondary clarification. To represent a clarification unit operation that includes bioburden reduction, a Sartopore® 2 final filter (0.45 µm) was used in-line as the third filtration stage.

Figure 1: Overview of a typical LV production workflow



Materials and Methods

LV production
LVs were produced using suspension-adapted HEK293 cells cultivated in a serum-free medium in a 10 L stirred-tank bioreactor. Cells were thawed from cryovials and sequentially expanded in shake flasks and seed bioreactors before inoculation of the 10 L production reactor.

Cultures were maintained at 37 °C with controlled pH and dissolved oxygen. Transient transfection was performed directly in the production bioreactor on day 2 using a plasmid mixture encoding the LV transfer vector, packaging constructs (Gag/Pol and Rev), and the envelope protein VSV-G. Transfection was carried out using the polyethyleneimine-based PEIpro® transfection reagent[8] at an optimized DNA: PEI mass ratio.

Following transfection, cultures were maintained at 37 °C without medium exchange to enable LV accumulation in the supernatant. One hour prior to harvest (48 h post-transfection), the culture broth was treated with nuclease to degrade residual DNA and reduce viscosity.

Infectious titers were determined using the Incucyte® system,[5] applying an inverse Poisson calculation as described by Figliozzi et al. (2016),[9] while total DNA and total protein concentrations were measured using PicoGreen® and Bradford assays, respectively.

Assessment of filter train configurations for LV clarification
Six filter configurations were evaluated to assess their ability to clarify LVs from crude cell broth. Each filter train consisted of two Sartopure® PP3 filters with decreasing retention ratings, followed by a 0.45 µm Sartopore® 2 filter. The specific filter combinations are summarized in Table 1.

To assess the impact of the 0.45 µm microfiltration filter on process performance, filter trains were also evaluated without this downstream filtration stage. Testing confirmed that inclusion of the Sartopore® 2 0.45 µm filter did not result in substantial loss of infectious titer, indicating that the filtration step did not compromise viral functionality under the tested conditions. Although the results primarily focus on the primary and secondary clarification filters, all data presented reflect filtration processes including the 0.45 µm microfiltration step.

Table 1: Overview of the six filtration configurations evaluated for clarification of crude LV production broth

Configuration	Filter 1 (primary clarification): Sartopure® PP3 | Capsule | Size 4		Filter 2 (secondary clarification): Sartopure® PP3 | Capsule | Size 4		Filter 3 (bioburden reduction): Sartopore® 2 | Capsule | Size 5	
	Retention rating [µm]	Area [cm^2]	Retention rating [µm]	Area [cm^2]	Retention rating [µm]	Area [cm^2]
1	50	180	1.2	130	0.45	300
2	20	180	1.2	130	0.45	300
3	8	180	1.2	130	0.45	300
4	50	180	0.65	130	0.45	300
5	20	180	0.65	130	0.45	300
6	8	180	0.65	130	0.45	300

Results

Filter preparation

Filters were assembled and flushed with ultrapure water (Arium® Pro) at ambient temperature using a constant flux of 300 LMH to remove particulates, as well as potential extractables and leachables. After flushing, filters were disassembled, gravity-drained, sealed in autoclave-compatible bags, and sterilized by autoclaving.

Sterilized filters were assembled into filter trains in a laminar flow cabinet. The assembled trains were conditioned with 450 mL of filter conditioning buffer at room temperature and incubated overnight. The conditioning buffer was subsequently removed by air blowout.

LV harvest filtration

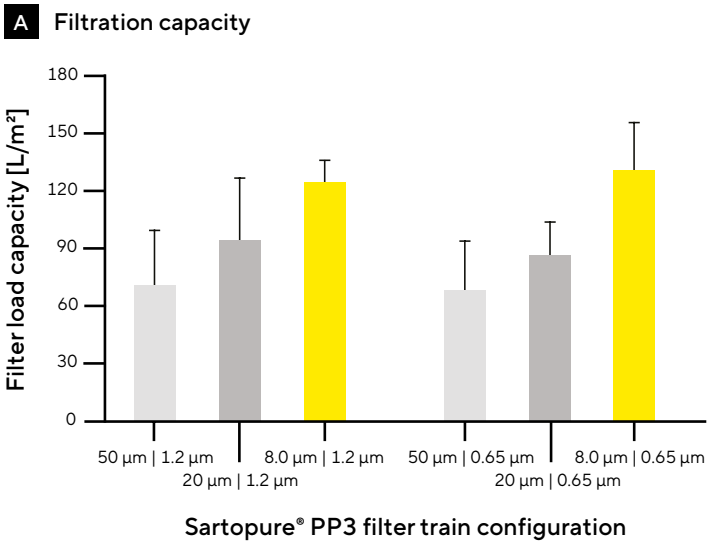
The LV-containing harvest was filtered at a constant flux of 200 LMH using the PendoTECH® Filter Screening System. The final filtrate was collected in 250 mL fractions, which were subsequently pooled for turbidity (NTU) and LV infectivity analysis. Each experiment was continued until the back pressure at any filtration stage reached 1.1 bar, which was defined as the termination criterion. Notably, this pressure limit is well below the maximum allowable operating pressure of 4.0 bar for the filters. The total filtrate volume processed at the termination point, normalized to the filtration area (L/m²), was defined as the load capacity of the respective filtration arrangement. LV broth remained stable and comparable across the entire experimental period; therefore, average feed value was used for normalization.

Filter capacity

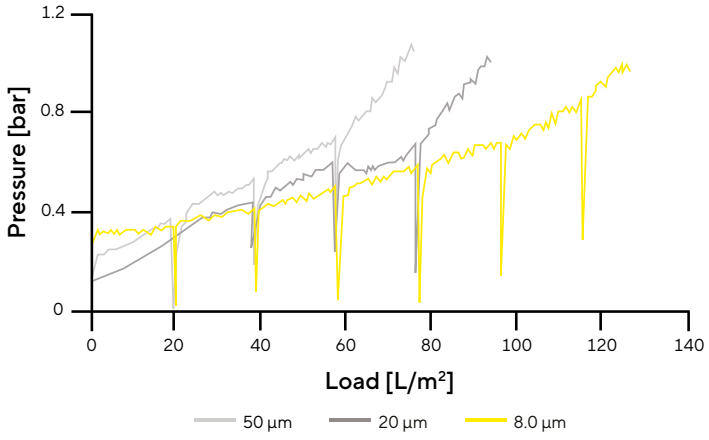
The filter load capacity (L/m²) determined for each filter combination is shown in Figure 2. Overall, filter capacity increased with decreasing retention rating of the primary filter. The configuration employing an 8 µm Sartopure® PP3 filter for primary clarification followed by either a 1.2 µm or a 0.65 µm filter for secondary clarification exhibited the highest load capacity. Compared to configurations using 20 µm or 50 µm primary filters combined with the same secondary filters, the load capacity was higher by factors of ~1.3 and ~1.8, respectively (Figure 2A).

The 8 µm filter demonstrated the slowest pressure increase over the course of filtration, reflecting its superior capacity for higher feed volumes before reaching terminal pressure, consistent with its optimized pore structure for LV clarification. In contrast, the 50 µm filter displayed the most rapid pressure rise, indicating earlier fouling and reduced load capacity under identical process conditions (Figure 2B).

Figure 2: Load capacity and pressure development of Sartopure® PP3 filter trains used for LV clarification. **(A)** Average load capacities determined for each serial filter combination (n = 3). Primary and secondary clarification were performed using Sartopure® PP3 filter trains; retention ratings are indicated on the x-axis. **(B)** Representative pressure profiles recorded upstream of the respective primary filters. Regular sharp pressure drops correspond to temporary pump stoppages for fraction container exchange.



B **Pressure increase across primary filter loads**

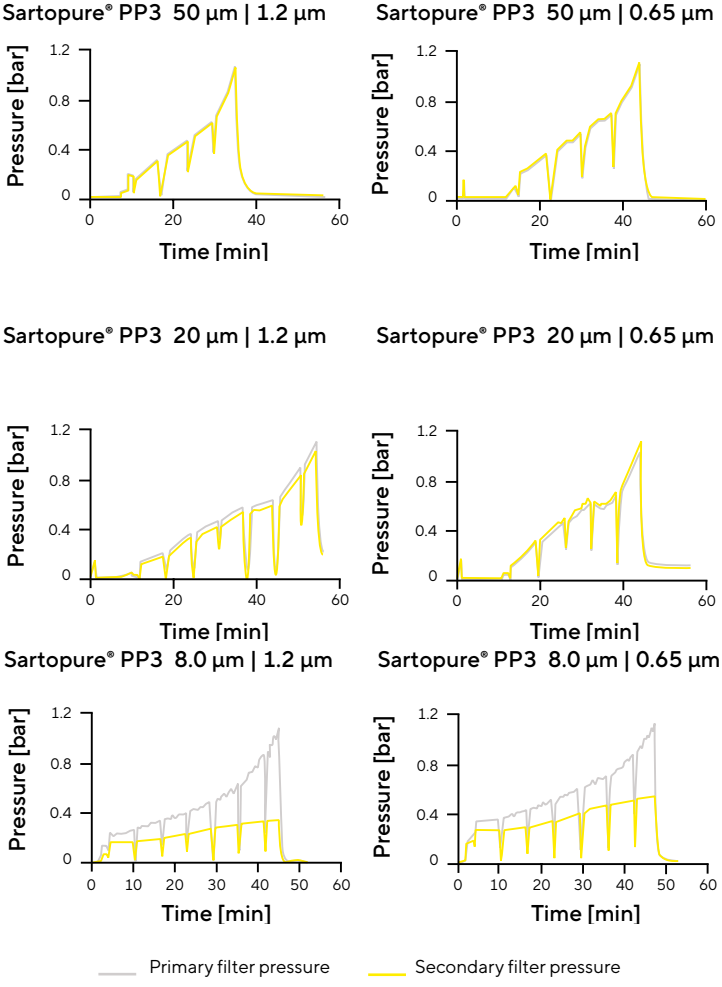


Detailed analysis of the pressure profiles measured upstream of both clarification stages highlights the superior performance of the configuration using an 8 µm filter for primary clarification (Figure 3).

With an 8 µm primary filter, back pressure across the secondary filters remained below ~0.45 bar for the 1.2 µm filter and ~0.5 bar for the 0.65 µm filter throughout filtration, demonstrating effective filtration and protection of the secondary filter. In contrast, use of 20 µm or 50 µm filters for primary clarification resulted in earlier loading of the secondary clarification filter, allowing fine particles and aggregates to pass through and leading to a faster rise in upstream pressure and earlier attainment of the 1.1 bar termination criterion (Figure 3).

Overall, the 8 µm primary filter effectively captures particles and aggregates, protects the secondary filter, and can be combined with both tested secondary filters without loss of filter capacity. However, the 20 µm and 50 µm configurations allow increased particle carryover, leading to accelerated loading of the secondary filter and a reduced overall filter capacity.

Figure 3: Pressure profiles measured upstream of both Sartopure® PP3 filters. Primary (50, 20, and 8 µm) and secondary (1.2 and 0.65 µm) filter retention ratings are indicated in each panel. Regular sharp pressure drops correspond to temporary pump stoppages for fraction container exchange.

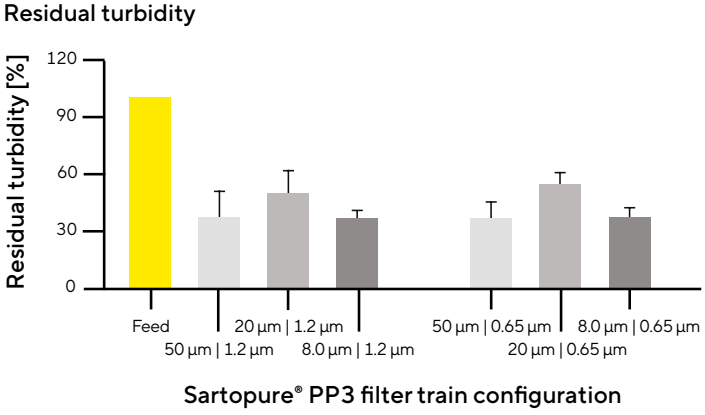


Turbidity reduction

Figure 4 shows residual turbidity for all filter combinations, expressed relative to the crude LV broth. Filter combinations using 50 µm and 8 µm Sartopure® PP3 filters for primary clarification, combined with either secondary clarification filter, showed a considerable turbidity reduction of approximately 65%. For the 20 µm Sartopure® PP3 filter combined with both secondary clarification filters, a robust turbidity reduction of approximately 50% was observed. As noted above, all filter trains included a Sartopore® 2 microfilter with a 0.45 µm retention rating, which is expected to provide sufficient particle removal for subsequent downstream steps. Accordingly, the absolute turbidity values obtained with the Sartopure® PP3 8 µm filter trains indicate a maximum of 65% turbidity reduction.

In summary, filter capacity and turbidity reduction results for configurations using an 8 µm Sartopure® PP3 primary filter in combination with either a 0.65 µm or a 1.2 µm secondary filter indicate a favorable combination for throughput and clarification performance.

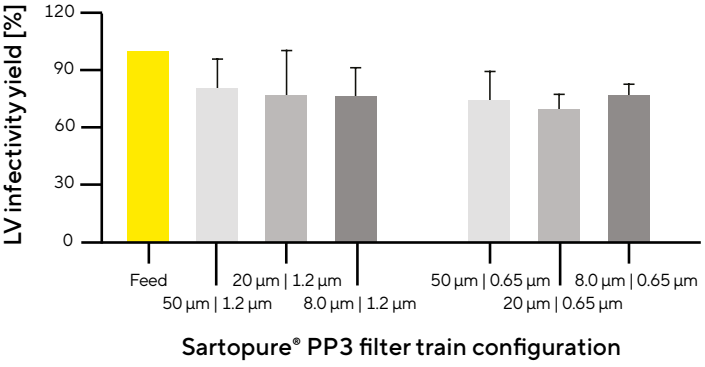
Figure 4: Residual turbidity expressed relative to the crude LV production broth (defined as 100%). Retention ratings of the Sartopure® PP3 filter trains are indicated on the x-axis.



Infectious titer

Figure 5 shows infectious titers measured in the filtrates obtained with the different filter combinations, expressed relative to the centrifuged LV broth. LV infectivity was preserved across all tested filter combinations, with relative infectious titers ranging from 70 – 106% and average values of approximately 70 – 80%, indicating effective retention of biological activity during filtration. The studies were performed using a pressure stop criterion of 1.1 bar and without a post-flush step; implementing this step would be expected to further increase infectious titer recovery. Overall, these results demonstrate that the filtration process preserved LV biological activity regardless of the filter combination, supporting robust downstream processing and consistent product quality.

Figure 5: Relative infectious titer after filtration, expressed as a percentage of the centrifuged LV production broth (100%). Retention ratings of the Sartopure® PP3 filter trains are indicated on the x-axis.



To evaluate the impact of the clarification setup on host cell impurity removal, total DNA and total protein concentrations were analyzed in the centrifuged feed material and the corresponding filtrates. The results in Table 2 provide a comparative overview of impurity levels across the tested filtration conditions from three independent experiments.

Total DNA and total protein content increased in the filtrates of all filter combinations compared to the centrifuged feed sample. This observation is explained by cell lysis during filtration than during benchtop centrifugation. The additional protein impurities introduced during clarification can be effectively removed during subsequent chroma-tographic purification steps, while residual host cell DNA can be efficiently reduced through nuclease treatment. As a result, these impurities do not represent a critical limitation to process performance. The enhanced clarification efficiency achieved outweighs the relatively minor increase in down-stream impurity clearance requirements, supporting the overall robustness and productivity of the process.

Table 2: Total DNA and total protein concentrations measured in centrifuged feed and corresponding filtrates obtained using different filter combinations

Config.	Sample name	Total DNA [ng/mL]	Total protein [µg/mL]
	Centrifuged feed	248.1	63.5
1	Sartopure® PP3 50 µm | 1.2 µm	521.8	206.4
2	Sartopure® PP3 20 µm | 1.2 µm	483.2	202.3
3	Sartopure® PP3 8 µm | 1.2 µm	619.0	272.3
4	Sartopure® PP3 50 µm | 0.65 µm	597.0	205.7
5	Sartopure® PP3 20 µm | 0.65 µm	476.4	242.7
6	Sartopure® PP3 8 µm | 0.65 µm	642.9	238.9

Discussion

All evaluated Sartopure® PP3 filter combinations achieved effective turbidity reduction (~65% with the 8 µm and 50 µm primary filters and ~50% with the 20 µm primary filter) while preserving LV infectivity (~70 – 80% relative to crude LV broth), providing sufficient protection for subsequent chromatography steps. However, the 8 µm configuration delivered superior throughput, with a 1.3- and 1.8-fold higher load capacity compared with the 20 µm and 50 µm variants, respectively, before reaching the defined termination pressure of 1.1 bar. This enhanced performance is attributed to the ability of the 8 µm primary filter to protect the finer downstream filter, thereby delaying secondary filter loading and extending overall system capacity. Across all evaluated performance parameters, results were independent of the secondary filter retention rating (1.2 µm or 0.65 µm).

Building on these findings, future work will focus on further optimization of the clarification step by evaluating different filter conditioning buffer solutions using the identified Sartopure® PP3 8 µm | 0.65 µm filter configuration.

Conclusion

This study demonstrates that a serial clarification strategy employing an 8 µm Sartopure® PP3 depth filter for primary clarification is a robust and effective approach for the clarification of crude LV production broths. Compared with 20 µm and 50 µm primary filters, the 8 µm configuration provides superior capacity due to improved protection of the secondary filter. Importantly, this performance was independent of the secondary filter retention rating, with both 1.2 µm and 0.65 µm depth filters showing comparable results.

The results highlight the importance of appropriate primary filter selection within multi-stage clarification concepts, as early particle retention strongly influences overall filtration performance and process efficiency. In particular, selecting a finer first-stage filter, as demonstrated by the 8 µm primary filter, enables stable operation of subsequent filtration stages and increases filter throughput.

By delivering a low-turbidity and biologically active feed to subsequent downstream operations, this clarification strategy provides a solid foundation for efficient, scalable, and consistent LV manufacturing.

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Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

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